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Meanderings of the mRNA through the Ribosome

Minireview

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Summary

A map of how mRNA travels through the ribosome is critical for any detailed understanding of the process of translation. This feat has recently been achieved using X-ray crystallography. The structure reveals, for the first time, details of the interactions between the mRNA and the 30S subunit beyond those at the tRNA binding sites. Elements of both 16S rRNA and ribosomal proteins contribute to mRNA binding. This work also identifies two tunnels that the mRNA passes through as it wraps around the 30S subunit. The mechanisms and mechanics of reading frame selection, translational fidelity, and translocation can now be informed by the structure.

In all cells, the templates that guide translation of the genetic code into protein products are messenger RNAs (mRNA), and the polymerase used to catalyze polypeptide synthesis is the ribosome. Protein synthesis requires efficient and accurate interaction of ribosomes with a multitude of cellular components, including mRNA and transfer RNA (tRNA). Translation occurs in a number of different steps, including initiation, elongation, and termination. While each of these stages requires the participation of different protein factors, mRNA and tRNA are much more ubiquitous components of the translational apparatus. Thus, an understanding of the interactions of mRNA and tRNA with ribosomes is minimally required to fully appreciate the process of translation. While there is an almost overwhelming amount of information regarding the interaction of tRNA with ribosomes and ribosomal subunits (see [1, 2] for reviews), much less is known about the interaction of mRNA with ribosomes. Now, Yusupova et al. [3] have directly mapped the path of an mRNA through 70S ribosomes and in so doing have brought together a large body of literature concerning positioning of the mRNA and maintenance of reading frame. Additionally, mapping the path of the mRNA reveals molecular features beyond the tRNA binding sites and thus has implications for all stages of translation.

The Players

While translation requires the precise interaction of a large number of components, an understanding of this process is further complicated by the complexity of ribosomes. All ribosomes are composed of two asymmetric ribonucleoprotein subunits; in prokaryotes the 70S ribosome (approximately 2.5 MDa) is composed of 30S and 50S subunits. Each subunit is responsible for different functions during translation of genetic information into protein products. The 30S (small) subunit is the site of translation initiation and participates in mRNA binding, tRNA selection, and codon/anticodon interactions. The 50S (large) subunit is responsible for catalyzing peptide bond formation, and interacts with the universally conserved amino acid-derivatized ends of the tRNAs.

Since tRNAs interact with both ribosomal subunits, ribosomes are often characterized in terms of three binding sites for this ligand. During translation elongation, a peptidyl tRNA is bound to the peptidyl (P) site of 70S ribosomes. A cognate aminoacyl tRNA is delivered to the aminoacyl (A) site, such that it base pairs with the appropriate mRNA codon. The exit (E) site is occupied by the deacylated tRNA that has just completed its role in translation. Following on these definitions, the portion of mRNA that is actively being translated can be defined as the P and A codons (see Figure 1). During translation initiation, the binding of the initiator tRNA appears to occur somewhat differently. Initiator tRNA is thought to bind directly to the P site of 30S subunits (not the A site of 70S ribosomes) and base pair with the start codon of mRNA. Thus, the binding and recognition events in initiation are intrinsically different than all of the subsequent tRNA binding steps. Regardless of the stage of translation, interaction of the ribosome bound mRNA with tRNA is critical for selection of cognate tRNA and selection and maintenance of the appropriate reading frame. A detailed understanding of the interactions of tRNA(s) and mRNA with the ribosome is essential for understanding cell physiology.

A Long Standing Question

The question of how the mRNA interacts with the ribosome is not a new question. In fact, the path of the mRNA through the ribosome has been studied by a variety of approaches ([3–5] and references therein). Perhaps, the most well known interaction between prokaryotic 30S subunits and an mRNA occurs between the 3′ end of small subunit ribosomal RNA (16S rRNA) and a sequence at the 5′ end of the mRNA. These two sequences base pair to form the Shine-Dalgarno helix [6]. This interaction is involved in initiation and determination of the correct reading frame in prokaryotes; however, this interaction is completely lacking in eukaryotic systems that have a much more elaborate initiation mechanism [7]. Nonetheless, the remaining mRNA/ribosome interactions are likely conserved across all kingdoms.

Prior to the recent advances in determining the structure of ribosomes and ribosomal subunits at high resolution [8–17], attempts to map the path of the mRNA were limited by the detail and accuracy of the available models for 30S subunits and 70S ribosomes. In some of these earlier attempts [4, 5], the mRNA wraps around the neck of the 30S subunit. The polarity of the mRNA

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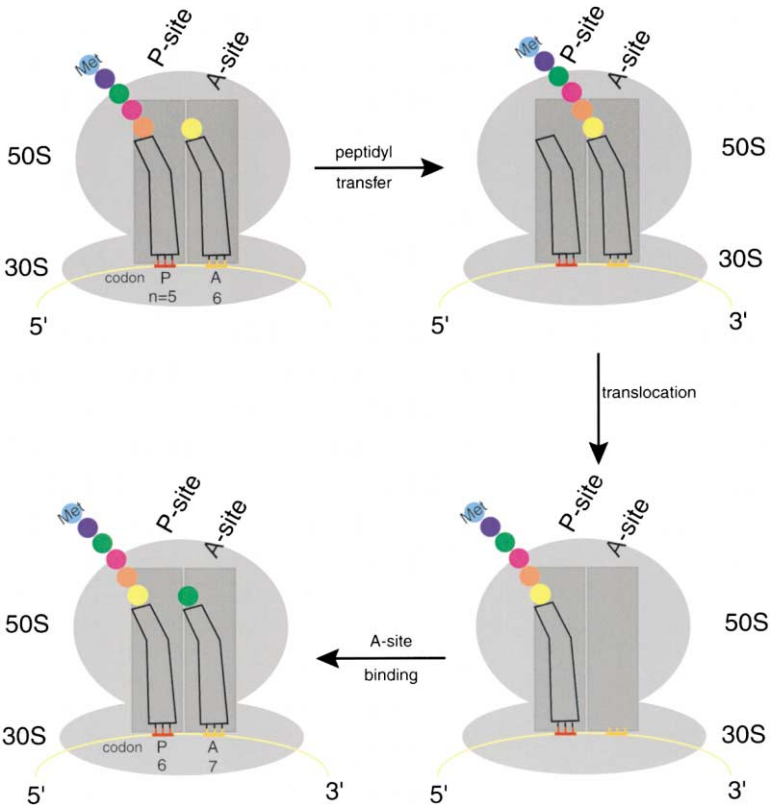

Figure 1. Simplified Schematic of the Translation Elongation Cycle

was fixed, in part, based on the mapped position of the 3′ end of 16S rRNA [18, 19], as being coincident with the 5′ end of the mRNA for the Shine-Dalgarno interaction to occur.

More recently, high resolution structural analysis has focused on the details of the interactions of the 30S subunit with the A codon and anticodon [16]. Because a minimal mRNA template of six nucleotides was used in that study, interactions beyond the A and P sites could not be observed. The P site interactions had previously been inferred based on mimicry of the P codon by 16S rRNA [9]. Together these studies identified the location and interations of the A and P codons within the 30S subunit but could not address the path of the mRNA outside of these functional sites.

A More Detailed Answer

To determine the path of the mRNA crystallographically, Yusupova et al. [3] took advantage of the demonstrated ability of ribosomes to bind certain tRNAs and, in fact, actually synthesize peptide bonds in the absence of a mRNA template [20, 21]. Therefore, difference density maps could be calculated from complexes containing mRNA and initiator tRNA and from those that contained only the tRNA to reveal density that corresponds to mRNA. Positioning the mRNA in these studies relied not only on difference density maps but also on the previously determined positions for the P and A codons (8). These positions were used to help correctly register the mRNA. Thus, the position of the mRNA upstream of the start site has been structurally mapped for the first time as has the region downstream of the A codon and the route that the mRNA traverses through the ribosome is revealed in some detail.

Overview of the mRNA Path

The mRNA appears to wrap around the 30S subunit (Figures 2 and 4), passing through two separate tunnels as it enters and exits the interface side of the subunit. The upstream tunnel is formed by portions of the head, platform, and neck of the 30S subunit, while the downstream tunnel is formed by portions of the head, body, and neck. These tunnels are formed by noncovalent interactions; therefore, one or both of the tunnels could open or change conformation during translation. These conformational changes may potentiate or be required for protein synthesis. Both tunnels appear to contain elements of 16S rRNA and small subunit proteins (see below and Figures 2–5).

The Shine-Dalgarno helix

The 5′ end of the mRNA (approximately position −15 in these studies [3]) is located on the solvent side of the 30S subunit (Figure 4). The Shine-Dalgarno sequence [6] is located near the 5′ end of the mRNA (Figure 2) and is poised to interact with the complementary sequence near the 3′ end of 16S rRNA (anti-Shine-Dalgarno). Density is observed that is consistent with base pairing between these two elements to form the Shine-Dalgarno helix and thus reveals the position of this important translation initiation element. This helix (shown in magenta in Figures 2–5) is accommodated in a cleft formed by 16S rRNA elements in the head, neck, and platform, along with platform proteins, particularly S11 (Figure 5a).

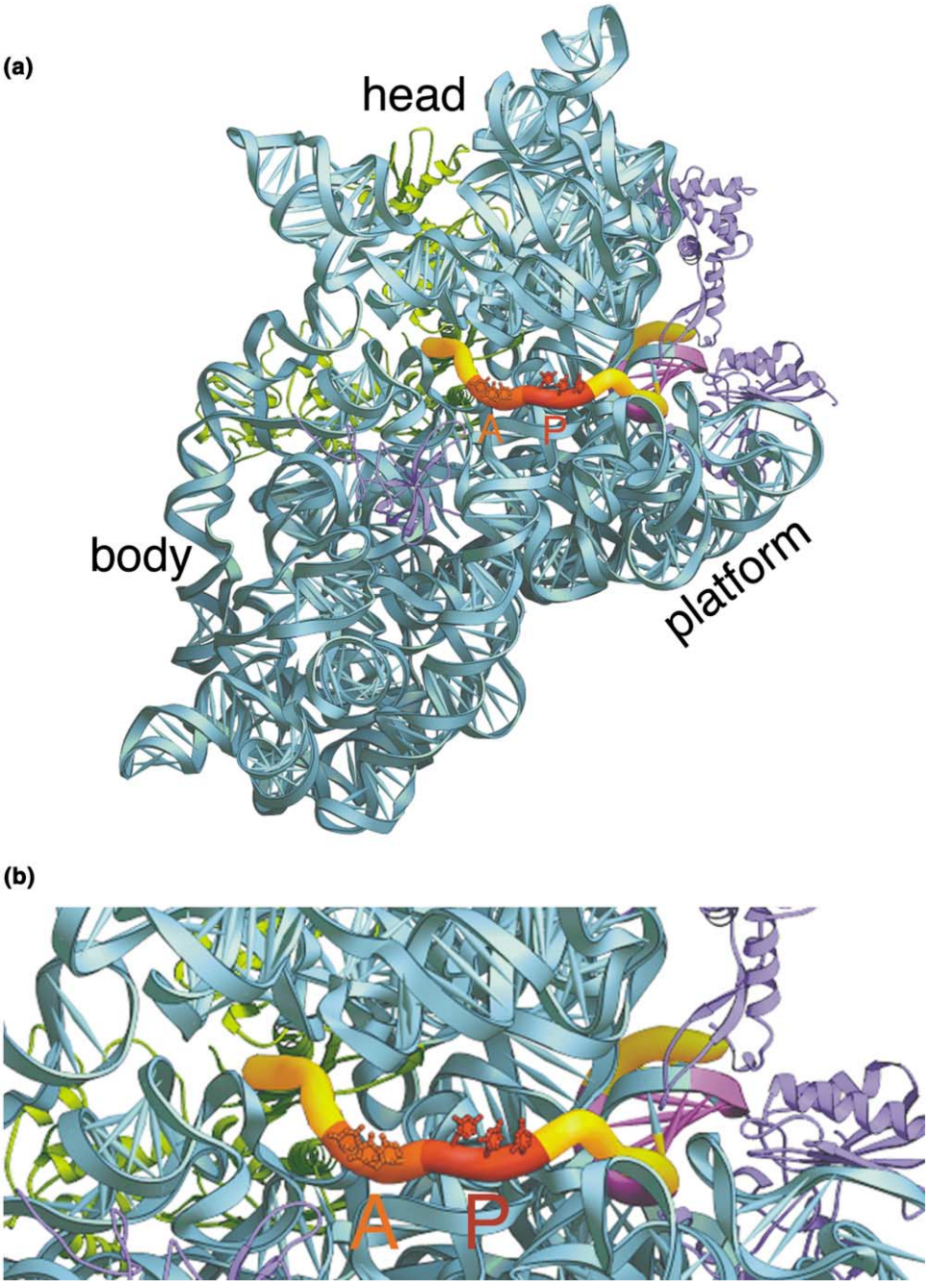

Figure 2. Path of the mRNA through the 30S Subunit

(a) General overview of the interface side of the 30S subunit. "A" and "P" indicate the A and P codons. Head, body, and platform are indicated. 16S rRNA is colored cyan, the Shine-Dalgarno helix is shown in magenta, the A and P codons are colored orange and red respectively, and the remainder of the mRNA is yellow. Ribosomal proteins S7, S11, and S12 are colored purple. Ribosomal proteins S3, S4, and S5 are colored light green. The remainder of the ribosomal proteins are omitted for simplicity.

(b) Close up of the mRNA path. Color scheme is identical to that described above.

The Upstream Tunnel

The mRNA from position −4 to −1 (therefore encompassing what would be the E codon) passes through a narrow tunnel so that the rest of the mRNA emerges on the interface side of the 30S subunit (Figures 4 and 5a). Standard codon-anticodon interactions are absent between the E site tRNA and the E codon; nevertheless, contact may still exist between the tRNA and mRNA in this site [8]. Ribosomal protein S7 from the head, elements of the 690 and 790 helices from the platform, and part of the neck all contribute to this tunnel (Figure 5a). This is consistent with the ability of nucleotides upstream of the start codon in an mRNA to be readily crosslinked to S7 [22,23] as well as with the suggestion

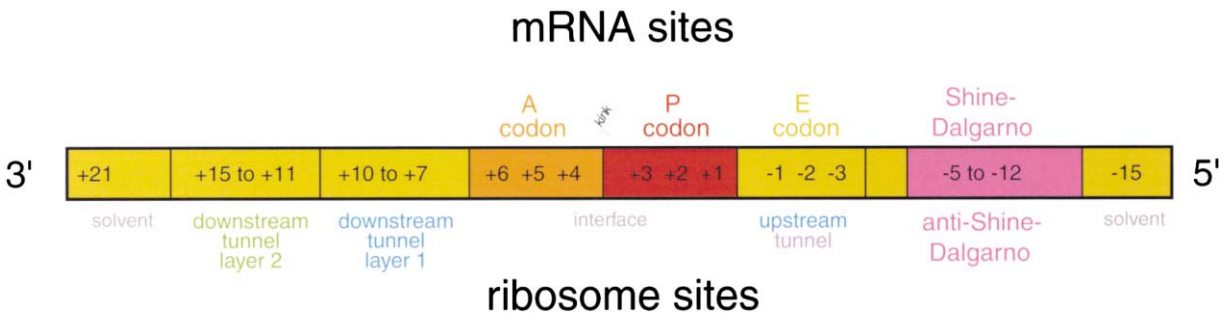

Figure 3. Schematic of a Model mRNA Illustrating Its Relevant Sites and Important Interactions with the Ribosome
Color scheme should reflect what is shown in Figure 2.

that S7 may actually block the exit path of the E site tRNA (8).

The P Codon

The P codon is found positioned as previously observed for a mimic [9] and is in a region that is conspicuously devoid of protein (Figures 2b and 5a). Nucleotide G926 has previously been implicated as playing a role at the P site [24–26], and the current work concurs with these findings. The authors suggest that G926 may play an active role in establishing a functional P site and therefore plays a role in initiation of translation. Other very highly conserved 16S rRNA residues, A790 and G791, along with U1498, play a role in the P codon interaction. Yusupova and colleagues [3] speculate that these nucleotides may move during ligand binding. This would be consistent with changes observed in cryoelectron microscopy upon subunit association [27].

As the mRNA makes the transition from the P to the A codon, its path is blocked. A phosphate causes the redirection of the mRNA, resulting in a kink between the P and A codons (Figures 2b, 3, and 5a). This kink was previously observed [8, 12, 16, 27] and is likely required to avoid steric clash due to the close approach of the tRNAs as they are accommodated at adjacent codons.

The A Codon

The Ramakrishnan [16] and Noller studies [3] are in good agreement with regard to A site interactions (Figures 2b and 5a). Along with nucleotides of the decoding site, including the universally conserved A1492 and A1493, G530 plays a role in A codon binding. The 530 loop is one of the most highly conserved regions in ribosomal RNA (28) and has been implicated in association of the A site tRNA [25, 28–34]. Moreover, G530 is specifically involved in EF-Tu-dependent binding of the A site tRNA [35], suggesting a role for this nucleotide in proofreading thought to occur at the A site (see review by [36]). Furthermore, these studies are consistent with much existing genetic and biochemical data regarding 16S rRNA nucleotides in the vicinity of the A site.

Unlike the P site where no ribosomal proteins are found, one ribosomal protein, S12, is positioned directly beneath A codon nucleotides +5 and +6 of the mRNA

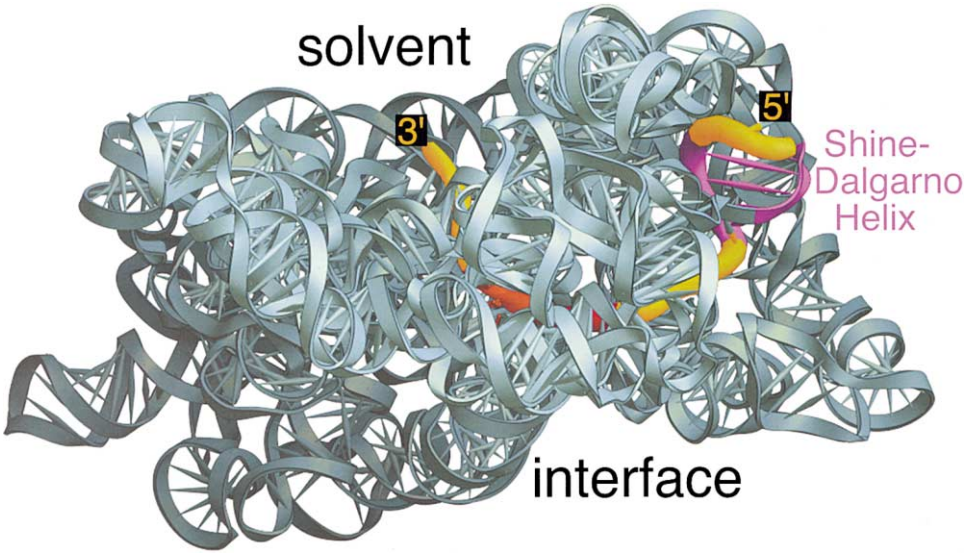

Figure 4. View from the Top of the Head of the 30S Subunit

16S rRNA and mRNA are colored as in Figure 2. All the ribosomal proteins are omitted for clarity. "5" and "3" correspond to the 5′ (−15) and 3′ (+15) positions of the mRNA. Solvent and interface correspond to surfaces of the 30S subunit. The Shine-Dalgamo helix is labeled.

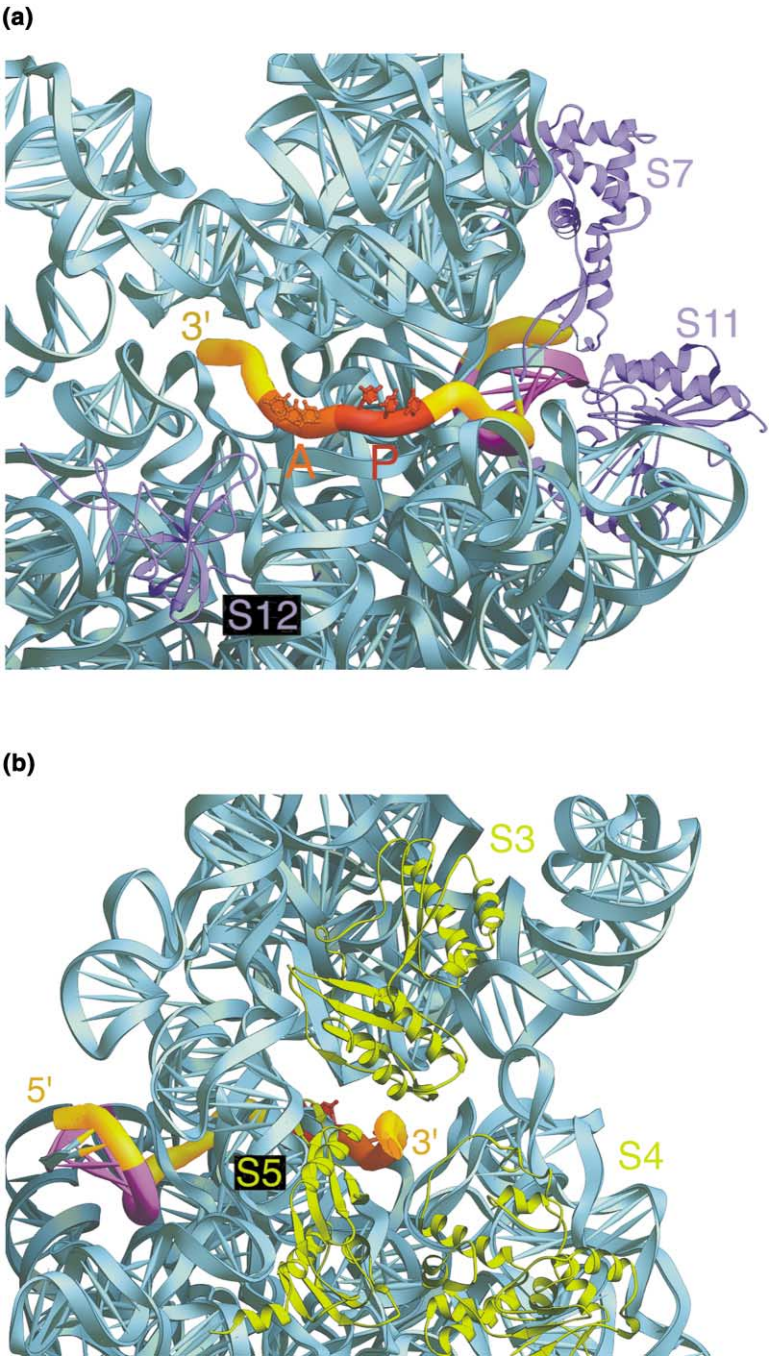

Figure 5. Detailed View of the mRNA Path through the 30S Subunit

Color scheme and lables are as described in Figure 2.

(a) Interface side view of the mRNA in the 30S subunit. Downstream tunnel proteins S3, S4, and S5 are omitted for clarity of the 16S rRNA (first) layer of the downstream tunnel.

(b) Solvent side view of the mRNA in 30S subunit. Upstream tunnel ribosomal proteins S7 and S11 are omitted for clarity of the upstream cleft.

(Figure 5a). Ribosomal protein S12 is the only protein that is located clearly on the interface side of the 30S subunit [9, 10]. This prominent and somewhat unique location suggests that S12 is poised to participate in functions of the interface. Reconstituted 30S subunits that lack S12 exhibit reduced levels of misincorporation of tRNAs [37], i.e., these ribosomes are hyperaccurate in the decoding process. Certain mutations in the coding region for S12 also yield similar hyperaccurate phenotypes [38, 39]. The position of S12 proximal to the A codon may explain how it influences accuracy of decoding. There is some discrepancy between different structural studies as to whether S12 is at the decoding site. Schluezen et al. [10] assert that the decoding site is devoid of protein, while two other studies [1, 16] clearly place S12 proximal to the A site. One possible explanation for this observed difference is that in the later studies, tRNA and model mRNAs were bound to the ribosomes or ribosomal subunits, such that these ligands were used to directly define the functional sites. However, the former study superimposes information from the 70S ribosome structure [12] onto their 30S subunit structure to facilitate localization of functional sites. Additionally, conformational differences reported between

the 30S subunit and 70S ribosome structures [8] could account for these discrepancies.

The Downstream Tunnel

Downstream of the A codon, the mRNA passes through another tunnel on its way back to the solvent side of the 30S subunit (Figures 2b and 4). Such a structure has previously been observed in structures of 70S ribosomes [5, 12, 40] and in some 30S subunit structures [10], but it has been notably absent in other studies of 30S subunits [41, 42]. The potential function of the integrity of the tunnel in maintaining processivity during elongation has been discussed in detail [10]. Another possibility is that conformational changes about or within this tunnel are involved in translation initiation. The open conformation could accommodate mRNA binding during translation initiation, which would then explain the ability of circular mRNAs to be translated [43]. Some of the sites that are thought to have altered conformation upon tunnel closing [10] are the same sites whose reactivities are changed upon P site tRNA binding, 50S subunit association, *or* binding of certain antibiotics [44]. In fact, mRNA binding alone may not be sufficient to close the tunnel; a more advanced stage in the initiation process may be involved in tunnel formation. This mechanism of tunnel opening and closing could allow regulation of translation initiation. Interestingly, this downstream tunnel can be thought of as having two concentric layers (compare Figures 5a and 5b).

Layers of the Downstream Tunnel

The first layer of this tunnel, defined as that closest to the A codon, is formed of 16S rRNA (Figure 5a). Elements from the head of the 30S subunit that are part of the top of the downstream tunnel may help position the mRNA immediately 3′ of the A codon. This interaction could orient or align the mRNA for its pending movement into the A site. Elements close to the very 5′ end of 16S rRNA are also found in this tunnel. Mutations that alter the stability of a functional element at the 5′ end of 16S rRNA [45] have been shown to result in the accumulation of free 30S subunits [46], demonstrating a defect in initiation. This suggests that interactions between the 5′ end of 16S rRNA and mRNA may play an important role in the initiation stage of translation.

The second layer of the downstream tunnel, through which the mRNA passes before emerging on the solvent side of the 30S subunit, is formed by ribosomal proteins S3, S4, and S5 (Figure 5b). The interactions between these proteins and mRNA are mediated in a sequence-independent manner via the phosphate backbone of the mRNA and basic amino acid side chains of the proteins. Genetic analysis has suggested that S4 and S5 play a role in fidelity, since mutations in both can lead to marked increases in the error rate of translation [47–49]. Therefore, the proximity of S4 and S5 to an mRNA in the tunnel could explain how these proteins effect translational fidelity while being located some distance from the decoding site. Omission of S3, S4, or S5 from in vitro reconstitution of 30S subunits results in a decrease in tRNA binding efficiency, although no alteration in fidelity was observed in vitro [37]. It should be noted that for the particles lacking S3, a decrease in tRNA binding capacity was shown to be a result of a 30S subunit assembly defect and not due to a direct role for S3 in tRNA binding [50]. Therefore, S3 may be critical for forming these functional sites while not directly participating in function. Also, it is clear from the positions, interactions, and biology of S4, S5, and S12 that their role in fidelity represents a delicate balance that must be tightly monitored to assure reasonable speed and accuracy during the course of translation.

Return to the Solvent Side

As the mRNA emerges from this tunnel, it is again exposed on the solvent surface of the 30S subunit, and thus, a path of the mRNA through the 30S subunit has been completely traced. Looking down on the top of the head of the 30S subunit (Figure 4), it is very evident that the mRNA begins at and returns to the solvent side of the 30S subunit. This study reveals for the first time that only the two codons (A and P) that are actively involved in that step of translation elongation are completely exposed on the interface surface of the 70S ribosome. It is likely that this positioning is relevant for translocation.

Functional Implications

As well as determining the path of the mRNA through the ribosome, Yusupova et al. [3] also suggest a manner in which multiple ribosomes may bind to the same mRNA. The arrangement of the ribosomes in the crystal lattice is such that the E site of one ribosome is immediately adjacent to the A site of another ribosome. This affords the possibility that the mRNA could thread directly from one ribosome to the next. While somewhat intriguing, the biological significance of this ribosome arrangement and possible mRNA threading remains to be seen.

How secondary structures within mRNAs are unwound during translation also remains unclear. Nevertheless, some mechanism for unwinding RNA helices and other structures must exist, since an RNA helix is too large to pass through the downstream tunnel. Noller and coworkers [3] suggest that an mRNA helicase could be an integral component of the 30S subunit. It is suggested that such an enzyme could be composed of S4 and S5 on the "bottom" and S3 on the "top" (Figure 4b). The two strands of the mRNA involved in forming the secondary structure could be separated by differential movement of the upper and lower portions of the downstream tunnel. The restrictive size of this downstream tunnel is also offered as an explanation for the role of pseudoknots in −1 frameshifting [51]. A pseudoknot would represent a major impediment to forward movement of the mRNA during translocation and thus could modulate the movement of the mRNA to dictate reading frame slippage.

Molecular Mimicry

One common theme that has appeared in many of the structures of ribosomes or ribosomal subunits is that ribosomal components can bind to functional sites in the absence of appropriate ligands. Noller and coworkers [3]

find that it is possible for an mRNA to fold back on itself to mimic the A site anticodon stem-loop. Carter et al. [14] found that the P site of one 30S subunit can be occupied by a portion of 16S rRNA from another 30S subunit in a manner that mimics the P site anticodon. It was also shown that the 3′ end of 16S rRNA can "fold back" and occupy the P and E sites to mimic mRNA codons [9]. Thus, in the vacant 30S subunit structures, the P site can be completely occupied even in the absence of any tRNA or mRNA. In a similar yet distinct interaction, it was found that the mRNA A codon can bind to its appropriate site even in the absence of an A site tRNA. Although the physiological consequences or significance of these interactions remains unclear, it is intriguing that the tRNA and mRNA binding sites appear to have a general propensity for occupation. This propensity must be balanced with the fidelity of codon/anticodon interactions required for accurate translation.

Remaining Questions

It is very clear that recent studies have made tremendous contributions not only to our molecular understanding of ribosome structure, but also to our understanding of RNA folding and RNA/protein interactions. However, it may be equally clear that our overall understanding of how the translational apparatus functions is still rather naive. The central question of how movement occurs during translocation still remains unanswered. Questions regarding molecular interactions during translation initiation and interaction with initiation factors, stability of codon/anticodon interactions during elongation, precise maintenance of the reading frame register, structural requirements for proofreading in combination with Elongation Factor-Tu, energetics of translocation and the role of Elongation Factor G, and the roles of different factors during termination are all yet to be answered. Nevertheless, with the powerful, ever emerging structural framework, the answers to these questions are closer than ever before. Indeed, with the work of Yusupova et al. [3] and many other recent structural studies of ribosomes and ribosomal subunits, the foundation now exists for understanding the dynamic events that occur during all stages of protein synthesis.

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